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THE CHEMICAL INVESTIGATION OF SOME GRAMINEAE OILS

By

Hannemon Ito

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Introduction

Although a large number of reports on the chemical studies of rice and barley, the important foodstuffs in this country, have already been made, there have been comparatively few reports on millet, (*Panicum miliaceum* L.), Japanese barnyard millet (*Echinochloa crusgalli* BEAUV. subsp. *edulis* HONDA var. *typica* HONDA) and Italian millet (*Setalia italica* KUNTH.) which rank with rice and barley as foodstuffs. As already reported[9] in the results of physico-chemical studies on proteins of the above mentioned three kinds of millets, the author had been engaged in the chemical investigation of these foodstuffs and his attention was then directed to the study of the oils of millet and Japanese barnyard millet in order to obtain further knowledge of them.

In 1887, KASSNER[10, 11] investigated millet oil and found ricinoleic, stearic, and millet oil acid ($C_{18}H_{32}O_2$) as fatty acids. Since then ANDREJEW[7] has also studied the same subject and recorded that erucic, oleic, ricinoleic and linolic acid are found in the saponifiable part of the oil. More recently the oil was investigated by DUNBAR and BINNEWIES[8], who reported that millet oil gave palmitic, oleic, linolic, iso-linolic, carnaubic and daturic acid through saponification.

On the other hand, an unsaponifiable substance was isolated by KASSNER[10, 12, 13] and DUNBAR and BINNEWIES[8] from millet oil.

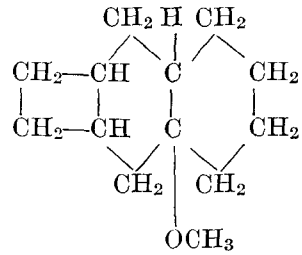
The former collected the crystalline substance which crystallised out on the bottom of the vessel when millet oil was left in a cold dark room, and designated it "panicole", melting at 285° (not corrected). It was stated to be optically inactive and to have the formula $C_{13}H_{10}O$. Under the action of hydroiodic acid in a sealed tube at 150°, methyl iodide was produced. By similar method, methyl chloride was obtained along with a solid substance which melted at 78° but could not be obtained in a crystalline form. The analysis gave the composition, $C_{12}H_{18}O$ for the latter.

On the oxidation of panicole with bromine and chromic acid in glacial acetic acid KASSNER obtained the products, $C_{13}H_{20}O_2$ and $C_{13}H_{18}O_5$ respectively. It was stated that the latter compound, panicolic acid, was a dibasic acid and has the formula,

$$C_{10}H_{13}\begin{cases}OCH_3\\COOH\\COOH\end{cases}.$$

Panicolic acid gave at 120–130° an acid anhydride, melting at 190°.

By the pyrogenetic treatment of panicole, hydrogen, acetylene and methane were obtained, and panicole was considered to be a hydrogenated naphthalene derivative, having the constitutional formula of



DUNBAR and BINNEWIES[8] recognised the pearly white crystals, m. p. 279° (corrected) gradually deposited from the oil extracted from the proso millet (*Panicum miliacum*) by ether or light petroleum. As this substance could not be identified, it was called "prosol". Prosol has the formula $C_{24}H_{36}O_2$ and gave negative tests for aldehyde, acid, carbohydrates, phenol, ester, anhydride, lactone or ketone groupings, but a positive acetylation for an alcoholic grouping. With hydroxylamine it produced an unsatisfactory quantity of a precipitate, presumably an oxime.

They concluded that prosol was an alcohol-ketone which was, in several respects, allied to the phytosterols.

The author has separated oleic, linolic, iso-linolic acid as unsaturated and palimitic, carnaubic acid as saturated from the saponifiable matters of millet oil. The carnaubic acid (m. p. 115.5–116.5° not corrected) was identified with its tribromoanilide, having a bromine content of 34.64 per cent. He has isolated a substance melting at 282–283° (not corrected) which corresponds to KASSNER's "panicole" or the "prosol" of DUNBAR and BINNEWIES. As a result of the close investigation of this substance, however, it has been proved to be quite different from "panicole" or "prosol", and the substance was named "miliacin" for which the formula $C_{32}H_{54}O$ has been assigned. According to the analytical results obtained miliacin is identical with sycoceryl alcohol ($C_{32}H_{54}O$) obtained by hydrolysis from sycoceryl acetate (named by DU LA RUE and MÜLLER in 1854) which was isolated from the resin of Fucus rubignosa by Edward H. RENNIE.[16] Yet these two substances seem to be quite different, since the melting point of sycoceryl alcohol, 114°, is much lower than that of miliacin. Miliacin has $[\alpha]_D^{25°} = +20.2°$ and the LIEBERMANN-BURCHARD's reaction of miliacin is slightly different from

that of phytosterol, for the former assumes a red violet which rapidly deepens to wine-red, while the latter shows a green colour. On treating miliacin with acetyl chloride in a sealed tube at 150° for three hours, white crystals are obtained from which, after several fractional recrystallisations from alcohol, two substances are isolated. One of them melts at 80–87° and the other at 112–114°.

The former compound was not further studied owing to the difficulty in obtaining it in a pure condition. It has no sharp melting point. The latter was, however, investigated and was shown to be a hydrocarbon of the composition $(H_5H_8)_6$ by analysis and molecular weight determination. These results appear to be interesting in that miliacin is closely related to isoprene which is considered to be genetically the nucleus of terpene. Therefore miliacin may be considered to bear a close relation to the terpenes. In order to examine accurately the function of oxygen in the miliacin molecule, first of all the methoxyl group was examined, with negative results. Acetyl chloride, anhydrous acetic acid, benzoyl chloride and thionyl chloride, were used under various conditions while in each case the unchanged original substance was recovered, showing it to have no hydroxyl group contrary to phytosterols.

On treating miliacin with phosphorus pentachloride, two kinds of chloride are secured, one of which crystallises out from alcohol, in rhombic hexahedrons and melts with decomposition at 238°, getting dark brown at 230°; the other melting at 220–222°, crystallising in prism form from alcohol. On analysis it was shown that these two products contain oxygen and have the compositions $C_{32}H_{51}Cl_3O$ and $C_{32}H_{53}ClO$ respectively. Accordingly the oxygen in miliacin could not be in a hydroxyl group but should be considered as an ether-shaped one.

Catalytic hydrogenation, platinum black or platinum oxide being used as a catalyser, showed that miliacin seems to be a saturated compound. By zinc dust distillation a liquid product was obtained together with a resinous substance having an aromatic odour. Although the yellowish liquid portion is likely to be a sesquiterpene, further investigation of the compound is still in progress.

The absorption spectra of miliacin were examined and general absorption found.

From unsaponifiable matters, a phytosterol was also separated, melting at 135–136° (not corrected) and having $[\alpha]_D^{25°} = -31.2°$, its acetate melting at 128° and having $[\alpha]_D^{25°} = -38.1°$. The absorption

bands of phytosterol were examined and this phytosterol found to contain a small amount of ergosterol. Another phytosterol melting at 130–133° was also isolated; it cannot, however, be regarded as a pure substance and its recrystallisation from alcohol is difficult. As to phytosterol, ANDERSON and his collaborator[1,2,3,4,5,6,15] have found various kinds of phytosterols with different melting points and optical rotatory powers, from plant oils. HEINRICH WIELAND and MITIZO ASANO[19] have obtained various kinds of sterols by preparing sterol benzoate from yeast. From these facts, the substance melting at 130–133° is considered to be a mixture of several kinds of sterols. *Syntherisma sanguinalis* DULAC var. *ciliaris* HONDA, *'mehishiba'*, is closely related to *Panicum miliaceum* L. from the botanical standpoint and was formerly classified in the same genera. The components of the oil of the former were also studied with nearly the same results as in the case of the latter, obtaining palmitic, oleic, linolic and carnaubic acid, miliacin and phytosterols.

Next, the author has investigated Japanese barnyard millet oil in the same way as in the case of millet oil with the following results. He has isolated only palmitic acid as a saturated fatty acid, and, as unsaturated ones, oleic, linolic and iso-linolic acids from the saponifiable matters. Moreover, from the unsaponifiable matters the substance melting at 280°, which is near the melting point of miliacin (m. p. 282–283°), has been isolated. But when mixed with a specimen of miliacin it melts at about 250°, which shows clearly that it differs from miliacin. The author thereupon proposes to name it "crusgallin" to distinguish it from miliacin. From the results of the analysis and the determination of molecular weight, the formula $C_{32}H_{54}O$ has been assigned to crusgallin. Judging from the molecular formula crusgallin should be considered as an isomer of miliacin. The general properties of crusgallin are similar to those of miliacin. Crusgallin has $[\alpha]_D^{25°} = +16.3°$, and is not effected by the hydrogenation procedure, using platinum black or platinum oxide as a catalyser, and shows a general absorption on the examination of absorption spectra. Crusgallin belongs to the monoclinic system and has perhaps a hollohedral symmetry in crystallographical observations as shown in the figure, measurement of which has been carried out by Mr. T. SHIGA, to whom the author expresses his hearty thanks. Further, from the unsaponifiable matters, the substance sparingly soluble in 95 per cent. alcohol was isolated, sintering at 62°, melting at 63° in white turbidity, and at 83° clearly. This substance, in spite of having a sterol colour reaction, seems to be allied to wax.

The purification of this substance is so difficult that the pure product could not be obtained. Its specific rotatory power is +1.8 at 25° in chloroform. Moreover, from the portion easily soluble in 95 per cent. alcohol a phytosterol, melting at 136.5–137.5°, was isolated and had $[\alpha]_D^{25°} = -21.3°$. Its acetate melted at 124–126° and has $[\alpha]_D^{25°} = -26.0°$. When a phytosterol of Japanese barnyard millet is mixed with that of millet, it melts at 136° without showing any depression. The absorption spectra of this phytosterol were examined and found to contain a small amount of ergosterol. From the portion extremely soluble in 95 per cent. alcohol, a substance melting at 91.5° (not corrected) was separated in needles, having $[\alpha]_D^{17°} = +61.9°$. From the results of the analysis and the determination of the molecular weight of this substance, crusgallin ($C_{32}H_{54}O$) may be considered as an isomeride because the difference of numerical values between the results falls within experimental error. The value of this substance, however, is also close to that of $C_{30}H_{50}O$ and $C_{29}H_{48}O$. Hence to which molecular formula this substance may be assigned will be determined by further investigation. This substance is named "hiyeol", for the sake of convenience. The absorption spectra of hiyeol were examined and general absorption found, as in the former cases.

Summarising the results of the present investigation, millet (*Panicum miliaceum* L.) oil and '*mehishiba*' (*Syntherisma sanguinalis* DULAC var *ciliaris* HONDA) oil are essentially composed of glycerides of the following fatty acids: oleic, linolic, iso-linolic, palmitic and carnaubic. From unsaponifiable matters, in addition to the presence of phytosterol (m. p. 135–136°), a white crystalline substance is obtained and designated "miliacin" ($C_{32}H_{54}O$) which is regarded as a new compound bearing a close relation to terpenes.

Next, Japanese barnyard millet (*Echinochloa crusgalli* BEAUV. subsp. *edulis* HONDA var. *typica* HONDA) oil is composed chiefly of glycerides of the following fatty acids: oleic, linolic, iso-linolic and palmitic. From unsaponifiable matters, besides a phytosterol (m. p. 136.5–137.5°), two new compounds are obtained which melt at 280° and at 91.5° respectively. To the former, crusgallin ($C_{32}H_{54}O$) is designated and to the latter hiyeol.

As above described, the author has isolated fatty acids from saponifiable matters and has also obtained sterols, miliacin, crusgallin and hiyeol from the unsaponifiable part of the oils of millet, Japanese barnyard millet and '*mehishiba*'. Furthermore, it is worthy of note

that the oils of these plants contain unsaponifiable matters besides phytosterols, which are identical or closely related substances. Miliacin is obtained from millet as well as *'mehishiba'*, whilst crusgallin from Japanese barnyard millet and these compounds have exactly the same molecular formula $C_{32}H_{54}O$, but are different. The author has shown that these special unsaponifiable matters are different from panicole or prosol described in the literature and has suggested that these are genetically closely related to the terpenes.

Experimental

Chapter I

On the Oil of *Panicum miliaceum* L. (Millet)

The material employed in this investigation was produced in the suburbs of Nagoya the preceding year, and was ground by means of a stone mill. 133.8 kilograms of ground material (moisture 13.0 per cent), on extraction by continuous percolation with hot benzine, yielded 5.2 kilograms of oil.

Some constants of the natural oil were determined, with the following results:

Specific gravity (15°)	0.933
Refractive index (30°)	1.467
Acid value	72.93
Saponification value	170.65
Iodine value	120.68
Hehner value	91.44
Reichert-Meissl value	1.10

The contents of products insoluble in acetone, unsaponifiable matters, and solid and liquid fatty acids in the oil were all determined by the following method:

On treating one part of the oil with about twenty parts of acetone a precipitate was produced, which was collected, dried in a vacuum over sulphuric acid, and weighed. From the portion soluble in acetone, the contents of unsaponifiable matters were estimated by Bömer's method; and the weight of unsaponifiable matters insoluble in 95 per cent. alcohol was also determined. This unsaponifiable matter and the part insoluble in acetone are crude miliacin. The contents of the solid and liquid fatty acids in the residual portion, after the unsaponifiable matters had been removed, were determined by the lead-soap-ether method. The analytical results were as follows:

	I	II
Subst. g.	18.00	15.41
Precipitate by acetone g.	0.3472(1.93%)	0.2794(1.81%)
Unsaponifiable matters g.	1.429 (7.92%)	1.118 (7.26%)
95% alcohol insoluble unsaponifiable matters g.	0.0377(0.21%)	0.0319(0.21%)
Solid fatty acid g.	1.719 (9.53%)	1.488 (9.84%)
Liquid fatty acid g.	13.22 (73.44%)	11.37 (73.75%)

Some constants of solid and liquid fatty acids thus obtained were measured with the following results.

Substances		Refractive index at 30°	Iodine value	Neutralisation value
Solid fatty acids	I.	—	16.90	209.59
	II.	—	18.29	207.81
Liquid fatty acids	I.	1.4552	145.99	196.38
	II.	1.4590	144.05	198.63

Separation of Miliacin and Phytosterol

One hundred gram batches of the oil and 200 c.c. of alcoholic potassium hydroxide solution, which was previously prepared by mixing 60 c.c. of potassium hydroxide solution, made by dissolving 200 grams of potassium hydroxide in water, the whole being made up to 300 c.c., and 140 c.c. of 95 per cent. alcohol, were put into a one litre flask. The contents of the flask were completely saponified on the boiling water bath for about an hour under the reflux-condenser, and were treated with 300 c.c. of water. This was introduced into a 2 litle separatory funnel containing 300 c.c. of water. In order to extract the unsaponifiable matters 800 c.c. of ether was poured into the separating funnel, which was shaken vigorously for about an hour. On allowing to stand for about half an hour, a clear sharp separation took place and the upper ethereal layer was separated from the lower. This extraction process was repeated until the upper ethereal layer did not finally assume a yellow colour.

In the course of this extraction, at the first treatment about 500 c.c. of ethereal solution were obtained, while at the succeeding treatment about 800 c.c. of the solution were secured.

On distilling off the ether from the solution which contained alcohol used for the saponification, a considerable amount of scaly crystals separated. These were collected while hot. The crystalline mass thus obtained was crude miliacin.

After keeping the filtrate in a cold dark room for about a week, a

great deal of the yellow crystalline mass was produced, which was separated and dried in a vacuum desiccator. This was treated with 95 per cent. alcohol and roughly separated into three parts; those readily soluble, those with difficulty soluble, and those insoluble in alcohol. In this case the former two parts consisted chiefly of sterols but the third part of miliacin.

The crude miliacin thus obtained was recrystallised from benzene and finally from xylene and was separated in colourless hexagonal plates, melting at 282–283° (not corrected).

The crude phytosterol being the part readily soluble in alcohol was recrystallised several times from 95 per cent. alcohol and colourless crystals were obtained which melted at 135–136° (not corrected). It was, however, so difficult to obtain a phytosterol in a pure condition from the part with difficulty soluble in alcohol that after several recrystallisations from alcohol the substance melted still indefinitely at 130–133° (not corrected). This gave a marked LIEBERMANN-BURCHARD reaction.

Another Method of Separating Miliacin from Phytosterol

This separation method was based largely upon the fact that miliacin is with difficulty soluble in cold acetone, while the oil and phytosterols are soluble in the solvent.

One hundred gram batches of the oil were introduced into about 1,000 grams of acetone, stirred for a while, and kept standing in the ice-chest for about 3 hours. The brown precipitate which settled on the bottom of the vessel was filtered and dried. This product was extracted with petroleum ether (b.p. 40–46°), using a Soxhlet apparatus for more than one week. After recovering petroleum ether, miliacin was crystallised out in colourless plates which, after several recrystallisations from benzene and then xylene, melted at 282–283° (not corrected) and was proved to be identical with the substance obtained by the above hydrolytic method, because there was no depression of the melting point when they were mixed.

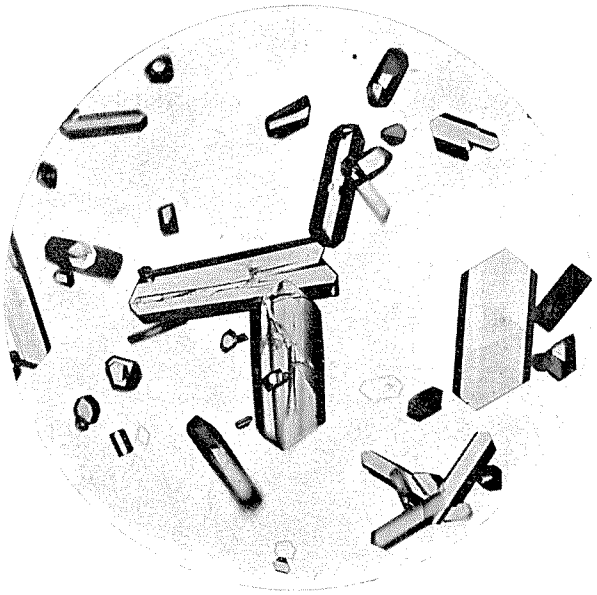
The oil, after miliacin had been mostly removed, was treated with alcoholic potash. Phytosterol was obtained and a small amount of miliacin.

In the first and the second isolation method, 3.9 kilograms of the oil had been used and 33 grams of the purest miliacin obtained, or 0.7 per cent. of the original oil. The total yield of the purest physosterol (m.p. 135–136°) was 5 grams or 0.13 per cent. of the original oil.

Properties of Miliacin

Miliacin, crystallises in hexagonal plates as is shown in the figure, is soluble in ether, chloroform, carbontetrachloride, xylene, benzene, sparingly soluble in methyl and ethyl alcohol, anhydrous acetic acid, ethyl acetate, acetone and insoluble in cold alcohol or water. Its melting point is 282–283° (not corrected).

Fig. 1



miliacin

Colour reaction:—About 5 miligrams of the substance were dissolved in 2 c.c. of chloroform, and 2 c.c. of conc. sulphuric acid were added slowly to the solution. The layer of chloroform was immediately represented by a white turbidity, while the layer of sulphuric acid was a yellow colour near the boundary of the two layers. But after a short time the layer of chloroform turned to a reddish colour. (HAEGER-SALKOWSKY).

A small amount of the sample was dissolved in anhydrous acetic acid, to which conc. sulphuric acid was added gently. The layer of the anhydrous acetic acid assumed a red-violet which after a short time changed into a wine-red, while the layer of sulphuric acid showed no colour. (LIEBERMANN-BURCHARD).

Specific Rotatory Power

The specific rotatory power of the substance was determined with the following result. (chloroform as a solvent).

$$[\alpha]_D^{25^\circ} = \frac{+1.32^\circ \times 100}{2 \times 3.262} = +20.2^\circ$$

Analysis

I.	Subst.	4.996 mg.,	CO_2	15,845 mg.,	H_2O	5.32 mg.,	C	84.53;	H	11.92%
II.	„	5.050 „	„	15.650 „	„	5.41 „	„	84.52;	„	11.99 „
III.	„	4.588 „	„	14.230 „	„	4.851 „	„	84.59;	„	11.83 „
IV.	„	4.606 „	„	14.255 „	„	4.945 „	„	84.41;	„	12.01 „
V.	„	3.826 „	„	11.880 „	„	4.100 „	„	84.68;	„	11.99 „
VI.	„	4.105 „	„	12.729 „	„	4.427 „	„	84.57;	„	12.07 „
VII.	„	0.1236 g.	„	0.3840 g.	„	0.1340 g.	„	84.73;	„	12.13 „
						Average	„	84.57;	„	11.98 „
						$C_{32}H_{54}O$ requires	„	84.50;	„	11.99 „

Molecular Weight

The determination of the molecular weight was carried out according to RAST's micro-method and the cryoscopic method, using benzene as a solvent, with the following results:

1. RAST's method.

Camphor 0.15 g., subst. 0.015 g., $\Delta t = 9^\circ$,

$$M = \frac{40 \times 100}{9} = 444.$$

$C_{32}H_{54}O$ requires 454.

2. Cryoscopic method

Subst. 0.05 g. in 19.45 g. benzene,
Depression of the freezing point: 0.030°,

$$M = \frac{5.12 \times 2.57}{0.030} = 439.$$

$C_{32}H_{54}O$ requires 454.

Catalytic Hydrogenation

Catalytic hydrogenation was carried out, using a mixture of 0.2701 gram of the substance, 15 c.c. of hexane and 0.1 gram of platinum black for about 24 hours, without revealing absorption of hydrogen. The recovered substance, after recrystallisation from benzene, melted at 282–

283° (not corrected) as in the case of the original substance. It was further mixed with the original substance, without depression of the melting point ensuing. The same treatment was tried, using platinum oxide as a catalyser, but the hydrogenation of miliacin did not take place.

Treatment with Benzoylchloride

A small amount of miliacin was dissolved in pure pyridine and a calculated amount of benzoylchloride added. The mixture was stirred for a while and kept in a cold room overnight. This was then introduced into a large amount of water and the resulting compound crystallised from benzene, when it separated in colourless plates, melting at 282–283° (not corrected). On mixing with the original substance no change in melting point was observed.

Treatment with Anhydrous Acetic Acid

One gram of the substance and 10 c.c. of anhydrous acetic acid were introduced into a tube and were heated at 150° for three hours after sealing the tube. Then the contents of the tube were introduced into cold water and crystallised from benzene. In this procedure though a small amount of the original substance was changed into a resinous matter, a large amount of it was not changed, since the depression of the melting point was not observed when it was mixed with the original product.

Treatment with Acetylchloride

Five grams of the substance and 50 c.c. of acetylchloride were introduced into a tube and heated at 150° for three hours after sealing the tube, when the contents of the tube were assumed to be rosetted. A small amount of unchangeable matter remaining in the tube was filtered, and from the filtrate excess of acetylchloride was removed under reduced pressure; 0.08 gram of unchanged substance, 1.5 grams of resinous matter and 2.92 grams of white plumelet-like crystals were subsequently obtained. On recrystallisation of these crystals, two needle-like substances, melting at 80–87° and at 112–114°, were separated. The former substance (m.p. 80–87°) was not considered to be pure. The latter (m.p. 112–114°) was analysed and proved to be a hydrocarbon as follows:

Subst. 4.140 mg., CO_2 13.417 mg., H_2O 4.385 mg.;
„ 3.989 „ , „ 12.927 „ , „ 0.257 „ ;

C 88.39, 88.38; H 11.85, 11.94%

$(C_5H_8)_n$ requires C 88.16, H 11.85%.

The molecular weight of the product analysed above was determined by the cryoscopic method with the following results. (benzene as a solvent).

Subst. 0.0818 g., benzene 17.43 g.

Depression of the freezing point (Δt) = 0.058°.

$$M = \frac{5.12 \times 4.69}{0.058} = 414,$$

$(C_5H_8)_6$ requires 408.

Treatment with Thionylchloride

To one gram of the substance was added 13 c.c. of thionylchloride and heated at 240° for 30 minutes. The product, after the mother liquor was driven off by suction, was washed with water and recrystallised several times from benzene, melting at 282–283°. In this treatment the original matter was not changed into an expected product.

Treatment with Phosphorus Pentachloride

To one gram of the substance 2 grams of phosphorus pentachloride were added, fused, and treated with a large amount of water. The resulting product was recrystallised several times from alcohol, and melted with decomposition at 238°, getting dark brown at 230°. The analytical results of this substance were as follows:

Subst. 4,399 mg., CO_2 11.060 mg., H_2O 3.620 mg.;
„ 4.585 „ , „ 11.520 „ , „ 3.750 „ ;
C 68.57, 68.52; H 9.21, 9.15%.
Subst. 3.218 mg., Cl 0.623 mg.;
„ 2.152 „ , „ 0.414 „ ;
Cl 19.36, 19.24%

$C_{32}H_{51}Cl_3O$ requires C 68.84; H 9.22; Cl 19.07%.

In this case a substance, which melted at 203° into a light brown-coloured jelly and turned to a brown liquid at 210° was also obtained as a by-product. It was analysed with following results.

Subst. 4.191 mg, CO_2 11.840 mg, H_2O 3.910 mg;
C 77.05; H 10.43%.

The chlorination of miliacin was next tried by adding phosphorus pentachloride into the saturated benzene solution. The resulting product could be separated by fractional crystallisation from alcohol into

two parts, one of them being easily soluble in alcohol, melting at 175–176° into a white milky state and at 183° into a clear liquid and showing needle shaped crystals the other being with difficulty soluble in alcohol, melting at 220–222° into clear liquid, and showing monoclinic hexagonal crystals. The analytical results of the latter substance were as follows:

Subst. 4.939 mg., CO_2 14.245 mg., H_2O 4.690 mg.;
„ 3.072 „ , Cl 2.226 „ ;
Found C 78.66; H 10.63; Cl 7.36%
$C_{32}H_{53}ClO$ requires C 78.55; H 10.93; Cl 7.25%.

The substance melting at 183°, contains about 3 per cent. of chlorine, which shows imperfect chlorination.

Determination of Methoxyl Group

According to the micro-ZEISEL method, the methoxyl group of miliacin was examined with negative result, rendering it evident that the methoxyl group was not present in the miliacin molecule at all.

Zinc Dust Distillation

A glass tube was filled as follows: A plug of glass wool was placed at the constricted end of the tube. This was followed by a 10 cm. layer of pumice about the size of a pea which had been impregnated with zinc dust. The coating of the pumice with zinc dust was accomplished as follows. A paste was prepared by rubbing up 100 grams of zinc dust with 30 c.c. of water. The pumice was added to this and the mixture stirred until the pumice was well covered with the zinc dust paste. The pumice was then removed from the paste and dried.

After the pumice-zinc dust layer was placed a mixture consisting of 4 grams of miliacin and 20 grams of zinc dust, to which some pumice impregnated with zinc dust had been added. Finally a 5 cm. layer of pumice-zinc dust was added.

The distillation experiment was begun by passing through the system a fairly rapid stream of dry hydrogen until all of the air was replaced. The current of the gas was then diminished so that only about three bubbles per second passed through. Heat was then applied under the front and rear ends of the tube containing the layers of pumice-zinc dust. Finally the mixture of miliacin and zinc dust was gradually heated until the temperature of the air was at 360°. Throughout the experiment the temperature was not permitted to exceed 360°.

Examination of the distillate:—The distillate consisted of a liquid portion together with a resinous substance. From 12 grams of the

original miliacin 5 grams of the resinous substance and 3 grams of a yellowish liquid substance were obtained, which, when distilled under diminished pressure, passed over between 92–98°/5 mm. and had an aromatic odour with the following specific gravity and refractive index:

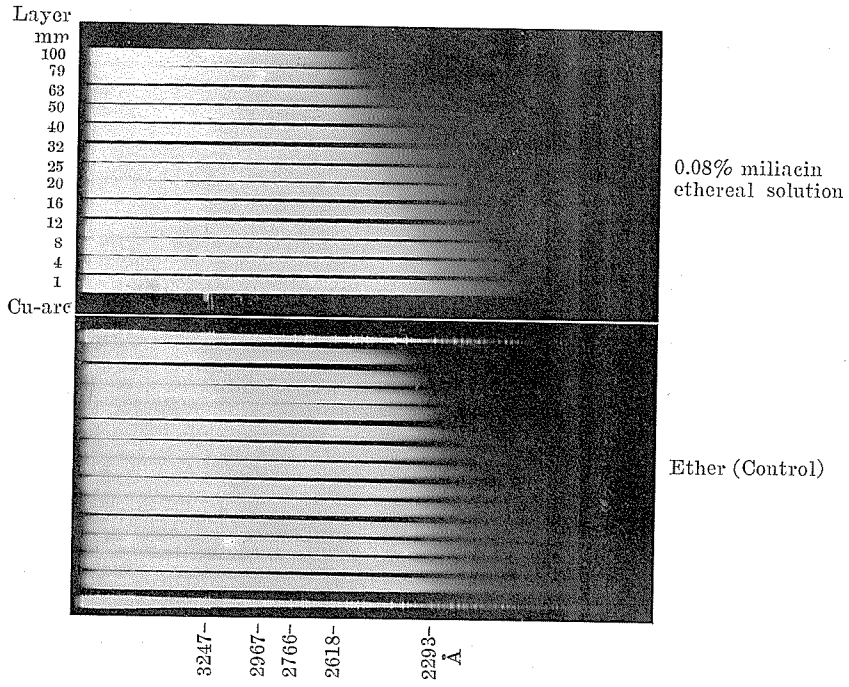
Sp. gr. 0.8988 at 18°; Specific refractivity 1.500 at 25°.

This substance is considered to be a sesquiterpene; further studies on it are in progress.

Examination of the Absorption Spectra

The absorption spectra of miliacin in ethereal solution were examined with an ADAM HILGAR E type quartz spectrograph, using a hydrogen GEISSLER tube as the source of illumination and showed general absorption as indicated in the following figure.

Fig. 2



Properties of Phytosterol

Phytosterol was soluble in ethyl alcohol, anhydrous acetic acid, xylene, benzene, ethyl acetate, ether, chloroform and carbon tetrachloride which melted at 135–136° (not corrected).

Colour reaction:—About 5 miligrams of the sample were dissolved in 2 c.c. of chloroform and 2 c.c. of conc. sulphuric acid added slowly. At that moment the layer of chloroform was represented by a white turbidity, while the layer of sulphuric acid was a yellow colour near the boundary of the two. But after a short time, the layer of chloroform turned to a reddish colour while the layer of sulphuric acid to a fluorescent green colour. (HAEGAR-SALKOWSKY).

A small amount of the sample was dissolved in anhydrous acetic acid, to which conc. sulphuric acid was added slowly. The layer of anhydrous acetic acid assumed a green colour, while the layer of sulphuric acid was a yellow colour near the boundary of the two. (LIEBERMANN-BURCHARD).

Specific Rotatory Power

Phytosterol, recrystallised from alcohol and dried at 110°, was dissolved in chloroform and the specific rotatory power was determined

$$[\alpha]_D^{25°} = \frac{-1.96° \times 100}{6.277 \times 1} = -31.2°.$$

Analysis

The product recrystallised from alcohol was dried at 110° under reduced pressure and lost in weight as follows:

Subst. 4.318 mg., loss in weight 0.092 mg., (2.16%).

The elementary analysis of this dried substance was carried out and the following results obtained:

Subst. 4.236 mg., CO_2 13.095 mg., H_2O 4.612 mg.

Found C 84.31; H 12.18%

Calc. for $C_{27}H_{46}O$ „ 83.86; „ 12.00 „.

Phytosterol Acetate

To obtain the phytosterol acetate, the phytosterol was dissolved in a small amount of anhydrous acetic acid, which was heated over a free flame for 2 hours. When it was poured into a large amount of water the acetate was separated, which after several recrystallisations from acetone, melted at 128°.

Specific Rotatory Power:—The specific rotatory power of this product was determined with the following results.
(chloroform as a solvent).

$$[\alpha]_D^{25°} = \frac{-2.05° \times 100}{5.375 \times 1} = -38.1°.$$

Analysis:—

Subst. 4.448 mg., CO_2 13.287 mg., H_2O 4.601 mg.
„ 4.081 „ , „ 12.213 „ , „ 4.240 „ .
Found C 81.48, 81.62; H 11.57, 11.62%
Calc. for $C_{27}H_{45}OCOCH_3$ „ 81.24 ; „ 11.29 %.

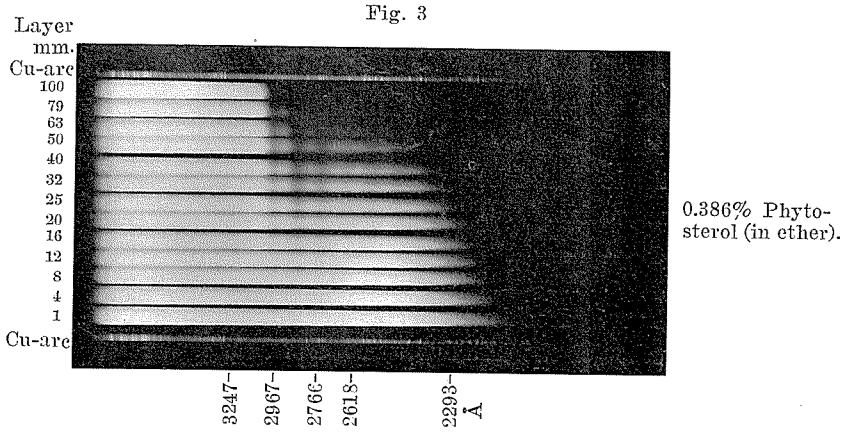
Determination of Acetyl Group:—

Subst. 4.085 mg., $N/100$-NaOH 1.005 c.c.,
„ 4.061 „ , „ „ 1.004 „ .
Found $COCH_3$ 11.8, 11.9%
Calc. for $C_{27}H_{45}O \cdot COCH_3$ „ 10.04%.

Determination of the Absorption Bands

The absorption bands of phytosterol in ethereal solution were estimated with an ADAM HILGAR E type quartz spectrograph, using a hydrogen GEISSLER tube as the source of illumination, and it was found that this phytosterol contained a small amount of ergosterol. The five absorption bands characteristic to ergosterol are shown in the figure (i.e. at 293, 282, 270, 260 and 250 $\mu.\mu.$).

Fig. 3



Separation of Solid and Liquid Fatty Acids

The solid and liquid fatty acids were separated from the previously mentioned saponifiable matters, which had been treated with ether for the removal of unsaponifiable ones by the lead-salt-ether method as follows:

On removing the alcohol as completely as possible, the saponifiable matter was dissolved in a large amount of water, and the solution was

made slightly acidic to litmus paper with acetic acid. To this solution was gradually added with constant shaking a proper amount of a filtered 10 per cent. solution of normal lead acetate, previously heated to boiling point. The flask was put aside to cool; when the lead soaps became hard and cold, the liquid was decanted off through a filter, and any of the lead salts left in the filter returned to the flask. The contents of the flask were washed many times with boiling water. Before pouring off the water, the mixture was carefully cooled each time by a rapid rotation of the flask under a stream of cold water. The flask was finally turned upside down and held over a filter by means of a clamp, till as much of the water as possible had drained off. To this dried lead salt a large amount of ether was added and the flask closed and thoroughly shaken, after which the contents of the flask were allowed to stand overnight, avoiding undue exposure to the air. Then it was centrifuged and repeatedly washed with ether till the layer of the ether showed no colour. By this procedure the lead salts of the liquid fatty acids were separated out from the salts of solid fatty acids. To the solution of the lead salt which had been brought to the separating funnel, was added an excess of hydrochloric acid (1:3). The separating funnel was then securely stoppered and vigorously shaken until lead chloride ceased to settle out. The solid lead salts insoluble in ether were transferred to the second separating funnel by means of a jet of ether. The mixtures in the funnel were treated with an excess of hydrochloric acid (1:3), decomposition of the lead salts being helped by vigorous shaking as in the case of liquid fatty acids.

On running off the aqueous solution, the ethereal solution was washed successively with small quantities of water until the washing water was free from hydrochloric acid, and dried over anhydrous sodium sulphate. The ether of the solution was then distilled off, and residual fatty acids were retained in a vacuum desiccator for further investigation.

From 3.9 kilograms of the natural oil 2.6 kilograms of liquid fatty acids and 0.3 kilograms of solid fatty acids were obtained.

Liquid Fatty Acids

To isolate the individual fatty acid from the liquid fatty acids which had already been obtained, the following experiments were carried out.

Liquid fatty acids were esterified with methyl alcohol, and the methyl esters were then subjected to fractional distillation under dimi-

nished pressure. Fractional distillation was repeated in each fraction and thus successive fractional distillations were made five times. In each fraction the iodine value, refractive index and saponification value of esters were determined, but satisfactory results could not be obtained.

Next, liquid fatty acids were subjected to distillation under diminished pressure, which passed over almost completely between 186–201°/2 mm. Using this distillate, the following bromination and oxydation experiments were carried out.

a) Bromination:—Twenty grams of the liquid fatty acids were dissolved in 200 c.c. of glacial acetic acid and to this bromine added so long as the colour of the bromine was not extinguished. During this procedure it was cooled with ice water. After standing overnight in the ice-chest, the bromides thus produced were introduced into a large amount of water and the resulting bromides were washed with water several times until no more bromine reaction was found. Then they were refluxed with hot petroleum ether, the portion soluble in petroleum ether being thus obtained. Next, the bromides insoluble in petroleum ether were treated with ether and it was found that all of them were dissolved in it. It was thus found that the di- and tetra- bromides were present, but hexa- or higher bromides were not present in them. The following experiments were then made on the bromides which were soluble in petroleum ether and in ethyl ether separately.

1) The bromides soluble in petroleum ether:—

The bromide was dissolved by a small amount of petroleum ether and placed overnight in the ice-chest. Thus the white crystals were separated on the bottom of the vessel and filtered off. This procedure was repeated several times until no more white crystals were separated out. The yellowish solution of petroleum ether was washed with dilute sodium thiosulphate solution to remove the free bromine. Then, to remove the moisture, it was treated with anhydrous sodium sulphate. After distilling off the petroleum ether, a yellowish brown liquid bromide was obtained which was analysed with the following results.

Bromine contents (B. C. method),

Subst. 0.5052 g., AgBr 0.4685 g.
„ 0.4277 „ , „ 0.3981 „ ,
Found 39.46, 39.61% of bromine;
Calc. for $C_{18}H_{34}O_2Br_2$ 39.15 „ „ „ .

Neutralisation value.

Found 121.4;

Calc. for $C_{18}H_{34}O_2Br_2$ 127.5.

From the above results, the yellowish brown liquid bromide should not be considered to be dibromostearic acid; from the neutralisation value and bromine contents, it seems to be dibromostearic acid mingled with another which has more bromine contents than itself.

2) The bromide insoluble in petroleum ether but soluble in ether:—

The portions of the white crystalline bromide, insoluble in petroleum ether but soluble in ether, were recrystallised from ether several times melting at 113.5–114.5° as is given in the literature. It was analysed with the following results.

Bromine contents (B. C. method):

Subst. 0.2127 g., 0.0992 g.; AgBr 0.2691 g., 0.1236 g.;

	Neutralisation value	Bromine %
Found	94.3	53.84, 53.04
Tetrabromostearic acid	93.5	53.27

From the above, the solid white crystalline bromide, insoluble in petroleum ether but soluble in ether, is tetrabromostearic acid.

b) Oxidation:—A certain amount of liquid fatty acids was taken and neutralised with 3 per cent. sodium hydroxide solution and introduced into two litres of water stirring vigorously to dissolve them. It was then slightly acidified with dilute acetic acid, cooled to 0°, and 2 litres of 1.5 per cent. potassium permanganate solution, previously cooled to 0°, was added from the burette with continuous stirring. Then it was kept overnight in the ice-chest. To remove the free potassium permanganate a saturated sodium sulphide solution was added to this. The resultant white mass was filtered, washed with water several times and dried over a $CaCl_2$ under diminished pressure. To remove the unoxidised oil, the dried mass was treated with petroleum ether, using SOXHLET's apparatus. The residue was refluxed with hot ether. The substance soluble in ether was recrystallised several times from alcohol, melting at 131–132°, and analysed with the following results.

Subst. 0.1101 g., CO_2 0.2758 g., H_2O 0.1143 g.;

	Neutralisation value	Molecular weight	C %	H %
Found	177.5	316.0	68.32	11.62
Calc. for $C_{18}H_{34}(OH)_2O_2$	177.4	316.3	68.29	11.47

The product thus obtained, as is seen from the above table, coincides

with dihydroxystearic acid. This fact shows that oleic acid is present in the liquid fatty acids.

The oxidised substance insoluble in ether was repeatedly recrystallised from alcohol and a substance obtained which melted at 173–174°, being identified as sativic acid. The substance was analysed.

Subst. 0.1035 g., CO_2 0.2358 g., H_2O 0.0990 g.;

	Neutralisation value	C %	H %
Found	159.7	62.13	10.70
Calc. for $C_{18}H_{32}(OH)_4O_2$	161.1	62.02	10.42

Considering the above bromination and oxidation, the following conclusions may be made.

1) Linolenic acid or higher fatty acids are not present in the liquid fatty acids.

2) The liquid fatty acids are composed largely of linolic, iso-linolic and oleic acids.

Solid Fatty Acids

To isolate the individual fatty acid from the mixed solid ones, the following procedure was carried out.

At first a fractional precipitation of fatty acids in alcohol was carried out by adding a small amount of water at a time, but satisfactory results were not obtained. Adding magnesium acetate to the alcoholic solution, fracional precipitation of the fatty acids was tried, but it was not fruitful. The solid fatty acids were then subjected to fractional distillation under 2 mm. pressure. The neutralisation values of the each distillate were measured and those distillates having nearly equal neutralisation values were united and further subjected to distillation. This treatment was carried out eight times successively. Each distillate was recrystalliesd several times from 70 per cent. alcohol; palmitic and carnaubic acids were isolated.

1) The one, which had a neutralisation value of about 220 after 8 redistillations, were recrystallised from 70 per cent. alcohol in lustrous scale like crystals melting at 62–62.5°. When it was mixed with an authentic specimen of palmitic acid, no change in the melting point was observed. Its neutralisation value was 218.9 and its analytical results were as follows:

Subst. 0.1051 g., CO_2 0.2893 g., H_2O 0.1195 g.;

Found C 75.07; H 12.72%;

Calc. for $C_{16}H_{32}O_2$ „ 75.00; „ 12.50 „.

Then it was esterified with methyl alcohol, and the resulting ester melted at 29.5–30.0°. It was found that the main fraction of solid fatty acids having the neutralisation value about 220, consisted of palmitic acid which must have been present in a considerably larger proportion.

2) On fractional distillation, the acids distilled over 200° under 2 mm. pressure, were collected and a further 8 times subjected to fractional distillation. Then it was recrystallised from hot 90 per cent. alcohol and the purest substance obtained, which melted at 73.5–74.5°. This acid was sparingly soluble in cold methyl alcohol, but easily soluble in ether, benzene, acetone, gracial acetic acid and hot alcohol. The substance was analysed.

Subst. 4.547 mg., CO_2 13.030 mg., H_2O 5.340 mg.;
„ 4.970 „ , „ 14.230 „ , „ 5.810 „ ;
Found C 78.15, 78.09; H 13.14, 13.08%;
Calc. for $C_{24}H_{48}O_2$ „ 78.18 ; „ 13.13%.

From the melting point, solubility and analysis, this acid was considered to be carnaubic acid. According to STÜRCKE[18], the melting point of this acid was 72.5°, according to MAYER and ECKERT[14], 74°, and to DUNBAR and BINNEWIES[8], 72.2°. It was treated with lead acetate and lead salt obtained after several recrystallisations from toluen, sintering at 111° and melting at 114–116°.

To obtain methyl ester from this acid, 0.1 gram of the acid was introduced into an excess of thionylchloride, which was heated at 100–120° in an oil-bath for about an hour. After the excess of thionylchloride had been driven off under reduced pressure, the chloride thus produced was introduced into methyl alcohol, which was recrystallised several times from ethyl acetate, sintering at 54° and melting at 56–57°. The tribromanilide of carnaubic acid was prepared according to the general method of ROBERTSON[17] as follows:—0.1 gram of the substance was added to an excess of thionylchloride which was heated between 100–120° on the oil-bath using a reflux condenser for about an hour. This was then treated with a little more tribromaniline than calculated, and was heated for about half an hour at 120–130°. The resulting product was recrystallised several times from alcohol and further from petroleum ether which melted at 115.5–116.5° (not corrected).

The bromine contents of this product were estimated:—

Subst. 4.501 mg., AgBr 3.770 mg.
Found 35.64% of bromine.
$C_{23}H_{47}CO \cdot NHC_6H_2Br_3$ requires 35.25 „ „ „ .

Solid fatty acids, from the above described results, appear to consist chiefly of palmitic acid and carnaubic acid but the former greatly predominating.

CHAPTER II

On the Oil of *Syntherisma sanguinalis* DULAC var. *ciliaris* HONDA (*'mehishiba'*)

The seeds of *'mehishiba'* were collected near Kasamatsu-chō in Gifu Prefecture in September, 1931. This plant grows in this country in the wilderness. The seeds were dried in sunlight, ground by means of a stone mill and the oil extracted from them with ether by continuous percolation. From 9 kilogram (moisture 12.56%) of the ground material 330 grams of the oil were obtained, which were 3.7 per cent. of the original material.

The general analysis of the ground material were as follows:

Moisture	12.56%
Ash	7.10 ,,
Crude fat	3.70 ,,
Crude protein ($N_2 \times 6.25$)	12.03 ,,
Total carbohydrate (glucose $\times 0.9$)	48.50 ,,

The unsaponifiable matters consisting largely of sterols and miliacin were estimated and solid and liquid fatty acids in the oil also determined by the following method: 14.7 grams of the oil were boiled with alcoholic potassium hydroxide for two hours, diluted with water and extracted five times with ether. The ether was distilled, and the residue, after it had been dried, weighed 2.47 grams, which cotained a considerable amount of miliacin in addition to sterols. Miliacin was obtained after treating the crude unsaponifiable matters with 90 per cent. hot alcohol, which weighed 0.39 gram. Through the lead soap 2.34 grams of saturated and 8.46 grams of unsaturated fatty acids were determined.

The percentages of each substance were as follows:

Unsaponifiable matters	16.75%
Miliacin	2.62 ,,
Solid fatty acids	15.92 ,,
Liquid fatty acids	57.50 ,,

Some constants of the natural oil, solid and liquid fatty acids were determined with the following results.

a) The natural oil.

Refractive index at 30°	1.4705
Acid value	104.5
Saponification value	178.8
Reichert-Meissl value	4.3
Polenske value	1.1
Iodine value	124.7
b) Solid fatty acids.	
Melting point	50–52.5°
Neutralisation value	207.4
Iodine value	11.3
c) Liquid fatty acids.	
Specific gravity 27°/27°	0.9024
Refractive index at 30°	1.4655
Neutralisation value	182.6
Iodine value	134.4

Preparation of Miliacin and Phytosterol

The oil, 300 grams, was saponified by heating on the water-bath with alcoholic potassium hydroxide. The soap solution was diluted with water and extracted with ether. After the distillation of ether, the residue was again boiled with alcoholic potassium hydroxide for two hours. On diluting the solution with water it was extracted with ether and the ethereal solution was washed, dried and filtered. On distilling off the solvent, a dark brown, crystalline residue was obtained which was treated with hot alcohol. Crude miliacin remained in a crystalline state. After several recrystallisations from benzene, 2 grams of miliacin were obtained in colourless hexagonal plates.

The unsaponifiable matters soluble in alcohol were treated with norite, recrystallised from alcohol several times and snow-white crystals obtained which melted at 135–136° and gave a marked LIEBERMANN-BURCHARD reaction. This crystalline substance was not enough in quantity for further investigations.

Miliacin thus obtained from 'mehishiba' melted at 282–283° (not corrected), and when mixed with the miliacin from millet oil no change in melting point was observed. Furthermore, the solubility, the rotatory power, and the colour-reactions were exactly the same as those of miliacin from millet oil.

$$[\alpha]_D^{16°} = \frac{+5.2° \times 100}{2.575 \times 1} = +20.1° \text{ (in chloroform)}$$

Miliacin from millet oil $[\alpha]_D^{25°} = +20.2°$

Analysis

1. Subst. 4.235 mg., CO_2 13.025 mg., H_2O 4.570 mg.
2. „ 4.180 „ , „ 12.881 „ , „ 4.524 „ .

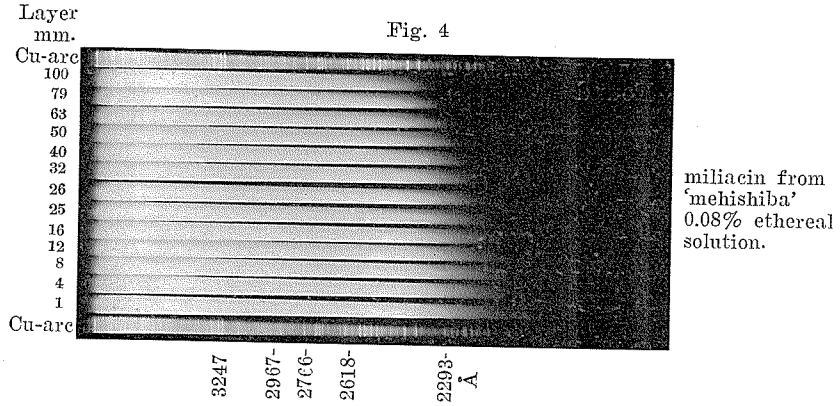
Found C 83.88, 84.04; H 12.08, 12.11%

$C_{32}H_{54}O$ requires „ 84.50 ; „ 11.98%.

Determination of the Absorption Bands

The absorption bands of miliacin in ethereal solution were estimated with an ADAM HILGAR E type quartz spectrograph, using a hydrogen GEISSLER tube as a source of illumination and showed general absorption as indicated in the following figure.

Fig. 4



The Liquid and Solid Fatty Acids

The isolation and the identification of the solid and liquid fatty acids were made by the same treatments as detailed in the case of millet. From 300 grams of natural oil 160 grams of liquid fatty acids and 45 grams of solid fatty acids were obtained.

On bromination of the liquid fatty acids di- and tetra- bromostearic acid (m.p. 114–115°) were produced, which shows the presence of oleic and linolic acids in the oil of *'mehishiba'*.

Analytical data.

Dibromostearic acid.

Bromine content (B. C. method).

Subst. 0.3244 g., AgBr 0.3013 g., Bromine 39.53%*

* It seems that the dibromostearic acid was contaminated with some compound whose bromine contents are greater than its own.

Calc. for $C_{18}H_{34}Br_2O_2$ 36.16 „

Tetrabromostearic acid.

Bromine contents (B. C. method).

Subst. 0.2658 g., AgBr 0.3323 g., Bromine 53.21%

Calc. for $C_{18}H_{32}Br_4O_2$ 53.28 „

From the solid fatty acids palmitic and carnaubic acids were isolated as in the case of millet oil.

Analytical data.

Palmitic acid.

Subst. 4.669 mg., CO_2 12.715 mg., H_2O 5.314 mg.

Found C 74.27; H 12.74%.

Calc. for $C_{16}H_{32}O_2$ „ 75.00; „ 12.50 „.

Carnaubic acid.

Subst. 4.171 mg., CO_2 12.018 mg., H_2O 4.972 mg.

Found C 78.58; H 13.34%.

Calc. for $C_{24}H_{48}O_2$ „ 78.18; „ 13.13 „.

CHAPTER III

On the Oil of *Echinochloa crusgalli* BEAUV. subsp. *edulis* HONDA var. *typica* HONDA (Japanese Barnyard Millet)

The material employed in this investigation was produced in Gifu Prefecture during the preceding year, and was ground by means of a stone mill, the oil being extracted by continuous percolation with hot benzine. From 48.8 kilograms (moisture 14.7%) of the ground material 2.5 kilograms of the oil were obtained, which constituted 5.1 per cent. of the original materials.

Some constants of the natural oil were determined and the following results obtained.

Specific gravity (15°)	0.914
Refractive index (30°)	1.467
Acid value	121.25
Saponification value	202.32
Iodine value	117.79
Hehner value	92.80
Reichert-Meissl value	0.52
Polenske value	1.4
Acetyl value	62.11

The products insoluble in acetone, unsaponifiable matters, solid and liquid fatty acids in the oil were determined by the following

method:—The oil was treated by twenty times its weight of acetone and a precipitate was produced, which was collected, dried in a vacuum over sulphuric acid, and weighed. From the portion soluble in acetone, the content of unsaponifiable matters were determined by BÖMER's method. The contents of the solid and liquid fatty acids in the residual portion from which had been removed the unsaponifiable matter were determined by the lead-soap-ether method. They were analysed with the following results:

Subst. g.	Precipitate by acetone g.	Unsaponifiable matter g.	Solid fatty acid g.	Liquid fatty acid g.
I. 18.08	0.3805(2.10%)	1.062(5.87%)	1.143(6.32%)	13.96(77.21%)
II. 16.79	0.3782(2.26%)	0.930(5.54%)	1.158(6.89%)	12.94(77.07%)

Some properties of the solid and liquid fatty acids thus obtained were as follows:—

Fatty acids		Refractive index at 30°	Iodine value	Neutralisation value
Solid	I.	—	14.47	213.48
	II.	—	15.06	213.42
Liquid	I.	1.4630	137.02	196.15
	II.	1.4630	138.48	195.62

Separation of Crusgallin, Hiyeol, and Phytosterol

One hundred gram batches of the oil were introduced into about 1,000 grams of acetone, stirred for a while and left stand in the ice-chest for about 3 hours. The resultant brownish precipitate was collected and dried in a vacuum desiccator. This product was extracted with a SOXHLET's apparatus, using petroleum ether (b. p. 40–60°) for more than one week. From 2.1 kilograms of oil, 4 grams of crude crusgallin were obtained. After the removal of the solvent from the portion soluble in acetone, the residue, from which the unsaponifiable matters were to be isolated, BÖMER's method was applied. On removing the ether from the extract which contained the unsaponifiable matters, the residual alcohol solution was kept in a dark room for about a week, when a great deal of the yellow mass was separated and dried in a vacuum desiccator. This material was treated with 95 per cent. alcohol and roughly divided into three portions, that easily soluble (I), that with difficulty soluble (II), and that insoluble (III) in alcohol.

After treating the portion (I) with norite and obtaining several recrystallisations from 95 per cent. alcohol, a small amount (about 0.5 g.) of a product was obtained, which crystallised in needles, melting at 91.5°.

This product was named "hiyeol" and is regarded as a new compound.

The portion (II) was repeatedly crystallised from alcohol and a phytosterol was obtained in thin hexagonal plates, melting at 136.5–137.5° (not corrected). The total yield of this product was about 5 grams. From this portion another product which was comparatively less soluble in alcohol than phytosterol was isolated. After several recrystallisations from alcohol, this product melted at 121–124°, weighed 2.4 grams, but was not pure enough as the melting point indicates.

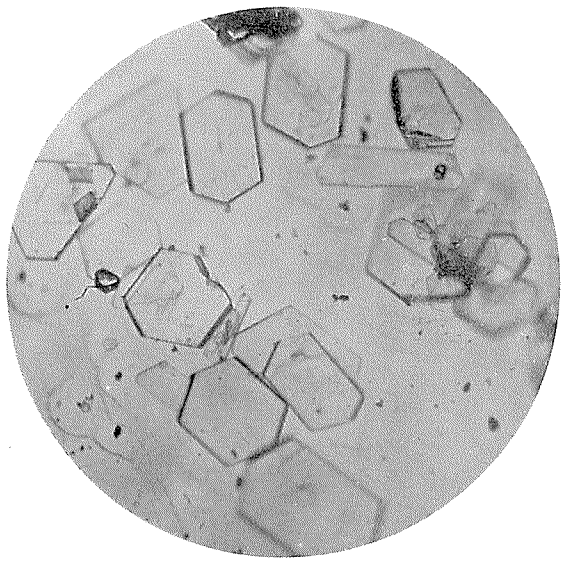
From the portion (III) insoluble in alcohol, and also from the above-mentioned crude crusgallin, after several recrystallisations from benzene, pure crusgallin was obtained which weighed 0.8 gram, melting at 280° (not corrected).

From this portion (III), also another product, through several recrystallisations from chloroform, was obtained, which weighed 1 gram, melting at 62–63°. This product may not be a pure substance, for it showed white turbidity at its melting point.

Properties of Crusgallin

Crusgallin crystallises in colourless hexagonal plates belonging to

Fig. 5



Crusgallin

the monoclinic sytsem as mentioned below, and melting at 280° (not

corrected). It is considered likely that crusgallin is different from miliacin, for when mixed with miliacin, a change in the melting point (about at 250°) was observed.

Crusgallin is soluble in ether, chloroform, carbontetrachloride, xylene, benzene, scarcely soluble in methyl and ethyl alcohol, anhydrous acetic acid, ethyl acetate, acetone; and insoluble in cold alcohol or water.

Colour reaction:—About 5 mg. of the substance were dissolved in 2 c.c. of chloroform, and conc. sulphuric acid added slowly to the solution; immediately the layer of chloroform was represented by a white turbidity, while the layer of sulphuric acid was a yellow colour near the boundary of the two layers. But after a while the layer of chloroform turned reddish, while the layer of sulphuric acid, a fluorescent green colour. (HAEGAR-SALKOWSKY's reaction).

A small amount of the substance was dissolved in anhydrous acetic acid, to which conc. sulphuric acid was added slowly. The layer of anhydrous acetic acid assumed a violet red which in a short time turned to a wine-red, while the layer of sulphuric acid showed no colour. (LIEBERMANN-BURCHARD's reaction).

Specific Rotatory Power

The specific rotatory power of the substance was determined with the following results. (chloroform as a solvent).

$$[\alpha]_D^{25°} = \frac{+0.44° \times 100}{1.350 \times 2} = +16.3°.$$

Analysis:—

Subst. 4.759 mg.,	CO_2 14.710 mg.,	H_2O 4.950 mg.;			
„ 5.002 „ ,	„ 15.490 „ ,	„ 5.290 „ ;			
Found	C 84.30, 84.46;	H 11.64, 11.83%;			
$C_{32}H_{54}O$ requires	„ 84.50;	„ 11.98 „ ;			
$C_{31}H_{52}O$ „	„ 84.47;	„ 11.90 „ ;			
$C_{30}H_{50}O$ „	„ 84.43;	„ 11.82 „ ;			
$C_{29}H_{48}O$ „	„ 84.39;	„ 11.73 „ ;			
$C_{28}H_{46}O$ „	„ 84.37;	„ 11.64 „ ;			

Molecular weight

The determination of the molecular weight was carried out according to RAST's camphor method and the cryoscopic method, with the following results:

1) RAST's method;
Subst. 0.015 g.; camphor 0.15 g.; $\Delta t = 9°$;

$$\text{Molecular weight} = \frac{40 \times 100}{9} = 444.$$

$C_{32}H_{54}O$ requires .....454.

2) Cryoscopic method;
Subst. 0.04 g.; benzene 21.25 g.
Depression of freezing point $(\Delta t) = 0.020°$

$$\text{Molecular weight} = \frac{5.12 \times 1.88}{0.020} = 481.$$

$C_{32}H_{54}O$ requires .....454.

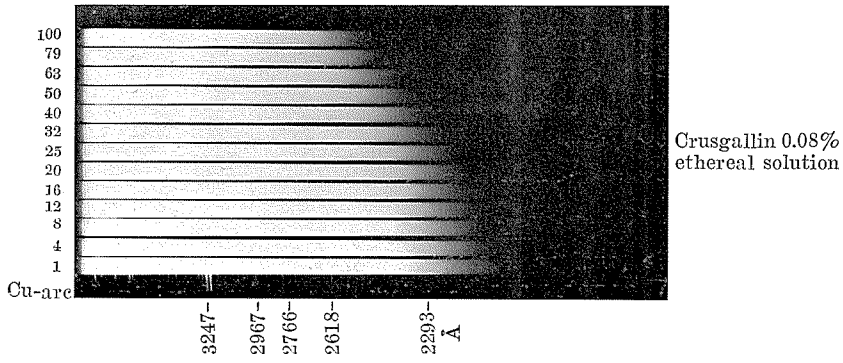
Catalytic Hydrogenation

Catalytic hydrogenation was tried for about 24 hours using a mixture of 0.1 grom of the substance, 15 c.c. of hexane and 0.1 gram of platinum black, only to discover no absorption of hydrogen. The recovered substance, after recrystallisation from benzene, melted at 280° (not corrected); when mixed with the original substance no depression of the melting point ensued.

Examination of the Absorption Spectra

The absorption spectra of crusgallin in ethereal solution were examined with an ADAM HILGAR E type quartz spectrograph using a hydrogen GEISSLER tube as the source of illumination. General absorption was found as indicated in the following figure.

Fig. 6



Crystallographic Studies

The author desires to express his hearty thanks to Mr. TAKEHIKO SHIGA, for his kindness in making the crystallographical observations.

Fifteen well developed crystals had been selected, from which eight crystals having clear reflecting images of the slit were chosen and V. GOLDSCHMIDT's two-circle goniometer was used. Then taking the largest crystal-face as the pole, ρ' and φ' of each face were measured, and the faces were projected by stereographic projection, from which the symmetry was examined. It was found that this crystal belongs to the monoclinic system and has perhaps a holohedral symmetry. Next, from the facts that the symmetry-plane of a monoclinic crystal is (010), the best-developed face was taken as the base and the next well-developed face as (100). From this assumption the numerical value of the angle β was found to be 99°13′. The values of ρ and φ were recalculated from the measured values of ρ' and φ' so as to take (001) zone as the fundamental circle of projection, and the faces were projected. The results are shown in the figure 7 and 8 and the following table.

The axial ratio was calculated, taking a pyramidal face of this crystal as the unit face e. g. ($\bar{1}11$) with the following results: $a:b:c=1.5110:1:1.49596$. Angles which were measured and calculated were as follows:

Crystal face	Calculated values ρ	φ	Observed values ρ	φ	n
001	*		9°13′	—	15
100	90°00′	90°00′	90°00′	90°00′	16
302	79° 1′	90°00′	79°00′	90°00′	5
111	*	*	79°13′	23°51′	17

The mark (*) shows the angles which had been used to calculate the axial ratios and n the numbers of the measured faces. The forms which appeared in all crystals were (001), (100) and ($\bar{1}11$), and the form (302) was rare.

A crusgallin crystal possesses two cleavages, one parallel to (001), more perfect, and the other parallel to (100).

The cleavage-flake of this substance is flexible, and the reflection of light by the crystal face was strongest on (001) and weakest on (302).

The horizontal stria on the face (100) are the traces of the cleavages parallel to (001).

Figure 8 shows an ideal illustration of the average habit and type of development of the crystal faces.

Fig. 7
Stereographic Projection of Crusgallin

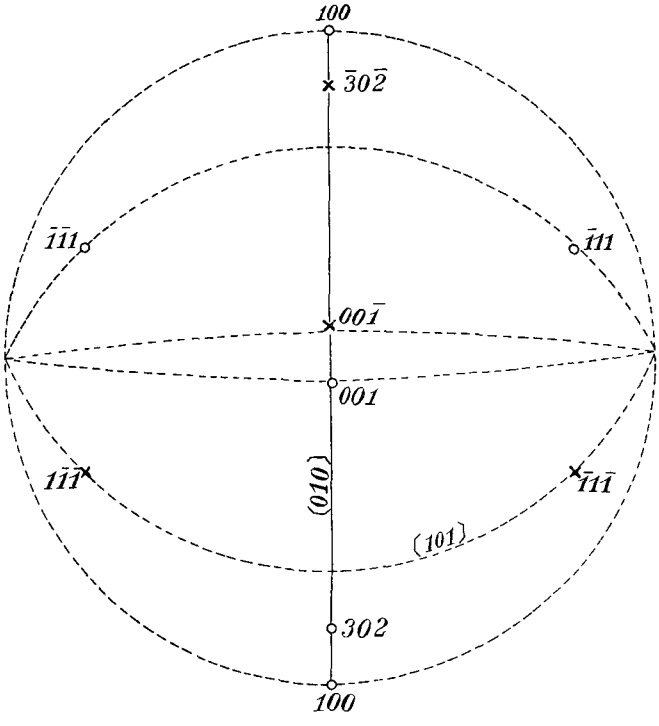
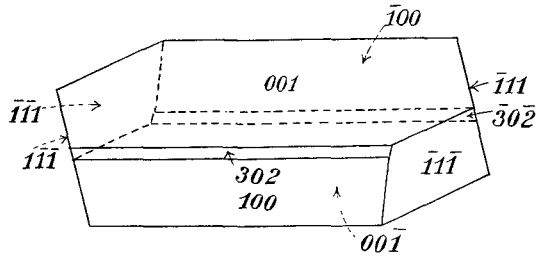


Fig. 8
Crystal Form of Crusgallin



Properties of Phytosterol

The substance is soluble in methyl and ethyl alcohol, anhydrous acetic acid, xylene, benzene, ethyl acetate, acetone, ether, chloroform and carbontetrachloride, and melts at 136.5–137.5° (not corrected). When it was mixed with a phytosterol from millet (*Panicum miliaceum* L.) the melting point was 136°.

Colour reaction:—About 5 miligrams of the substance were dissolved in 2 c.c. of chloroform to which 2 c.c. of conc. sulphuric acid were added gently. On that instance the layer of the chloroform was represented by a white turbidity, while the layer of sulphuric acid was yellow colour near the boundary of the two layers. But after a short time the layer of chloroform was changed into a reddish colour, while the layer of sulphuric acid changed to a fluorescent green colour. (HAEGAR-SALKOWSKY reaction).

A small amount of the sample was dissolved in anhydrous acetic acid, to which conc. sulphuric acid was added gently. The layer of anhydrous acetic acid assumed a green colour, while the layer of sulphuric acid was a yellow colour near the boundary of the two layers. (LIEBERMANN-BURCHARD reaction).

Specific Rotatory Power

The compound, melting at 136.5–137.5°, was dried at 110° and dissolved in chloroform. The specific rotatory power of this solution was determined with the following results.

$$[\alpha]_D^{25°} = \frac{-1.07° \times 100}{4.862 \times 1} = -22.0°$$

Analysis

The substance recrystallised from alcohol was dried at 110° under reduced pressure and lost in weight about 3 per cent.

Subst. 4.475 mg., Loss in weight 0.137 mg. (3.06%).

The anhydrous substance was analysed.

Subst.	0.1004 g.,	CO_2 0.3074 g.,	H_2O 0.1078 g.
„	0.1062 „,	„ 0.3252 „,	„ 0.1152 „.
„	4.338 mg.,	„ 13.368 mg.,	„ 4.725 mg.
Found	C 83.50, 83.51, 84.04;		H 12.01, 12.14, 12.19%.
Calc. for $C_{27}H_{46}O$	„ 83.86;		„ 12.00 „.

Molecular Weight

The determination of the molecular weight of this product was carried out according to RAST's camphor method with the following results:

Subst. 8.2 mg., Camphor 84.6 mg., $\Delta t = 10°$;

$$\text{Molecular weight} = \frac{40 \times 970}{10} = 388.$$

Calc. for $C_{27}H_{46}O$ 386.

Phytosterol Acetate

The phytosterol dissolving in a small amount of anhydrous acetic acid was heated over a free flame for 2 hours. On pouring it into a large amount of water, the acetate was separated and recrystallised from ethyl acetate, melting at 124–126° (not corrected).

Specific Rotatory Power:—The specific rotatory power of this product was determined in chloroform.

$$[\alpha]_D^{25°} = \frac{-1.44° \times 100}{5.550 \times 1} = -26.0°$$

Analysis:—

Subst. 4.173 mg., CO_2 12.513 mg., H_2O 4.405 mg.;
„ 4.263 „ , „ 12.791 „ , „ 4.444 „ ;
Found C 81.78, 81.83; H 11.81, 11.67%;
Calc. for $C_{27}H_{45}O \cdot COCH_3$ „ 81.24; „ 11.29%.

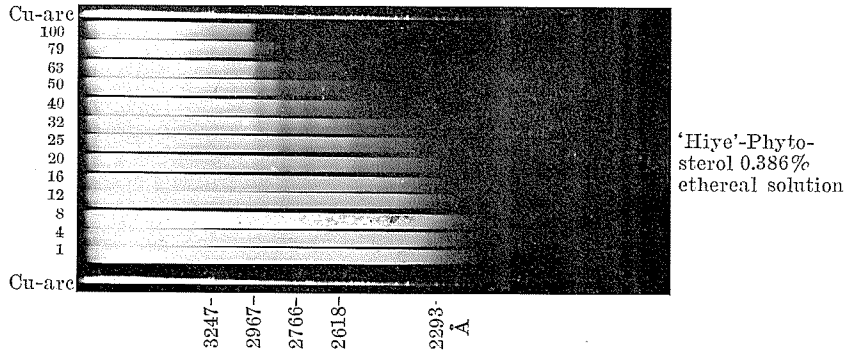
Determination of Acetyl Group:—This determination was carried out after the method of PREGL and SOLTYS.

Subst. 4.002 mg., $N/100$ ×1.118 NaOH 0.97 c.c.
„ 3.892 „ , „ „ 0.94 „ ;
Found CH_3CO 11.7, 11.6%,
Calc. for $C_{27}H_{45}O \cdot COCH_3$ „ 10.04%.

Examination of the Absorption Spectra

The absorption spectra of the phytosterol in ethereal solution were examined with an ADAM HILGAR E type quartz spectrograph, using a hydrogen GEISSLER tube as the source of illumination; it was found that

Fig. 9



this phytosterol contains a small amount of ergosterol for the five absorption bands shown in the figure are characteristic of ergosterol (i.e. at 293, 282, 270, 260 and 250 $\mu.\mu.$).

Properties of Hiyeol

Hiyeol is very readily soluble in methyl and ethyl alcohol, acetic acid, anhydrous acetic acid, ethyl acetate, acetone, ether, chloroform, benzene, xylene and carbon tetrachloride, and crystallised out from alcohol in needles, melting at 91.5° (not corrected).

Colour reaction :—A small amount of the substance was dissolved in anhydrous acetic acid, to which conc. sulphuric acid was added with caution. The layer of anhydrous acetic acid assumed a deep red colour.

Specific Rotatory Power

Hiyeol was dissolved in a chloroform and a determination of its specific rotatory power gave the following result:

$$[\alpha]_D^{27°} = \frac{+1.9 \times 100}{3.08 \times 1} = +61.9°.$$

Analysis

The product, recrystallised from alcohol, was analysed and the following results were obtained.

Subst. 4.802 mg., CO_2 14.860 mg., H_2O 5.030 mg.
„ 5.090 „ , „ 15.740 „ , „ 5.420 „ .

Found		C	84.40, 84.34;	H	11.72, 11.92% ;
$C_{32}H_{54}O$	requires	„	84.50;	„	11.98 „ ;
$C_{31}H_{52}O$	„	„	84.47;	„	11.90 „ ;
$C_{30}H_{50}O$	„	„	84.43;	„	11.82 „ ;
$C_{29}H_{48}O$	„	„	84.39;	„	11.73 „ ;
$C_{28}H_{46}O$	„	„	84.37;	„	11.64 „ .

Molecular Weight

The molecular weight of hiyeol was determined according to RAST's camphor method and the cryoscopic method with the following results:

1) RAST's method :—

Subst. 0.016 g., camphor 0.15 g., $\Delta t = 10°$;

$$M. = \frac{40 \times 106}{10} = 424.$$

2) Cryoscopic method:—

(i) Subst. 0.1036 g., benzene 21.57 g., $\Delta t=0.056°$;

$$M.=\frac{5.12\times 4.803}{0.056}=439.$$

(ii) Subst. 0.1728 g., benzene 14.92 g., $\Delta t=0.140°$;

$$M.=\frac{5.12\times 11.58}{0.140}=423.$$

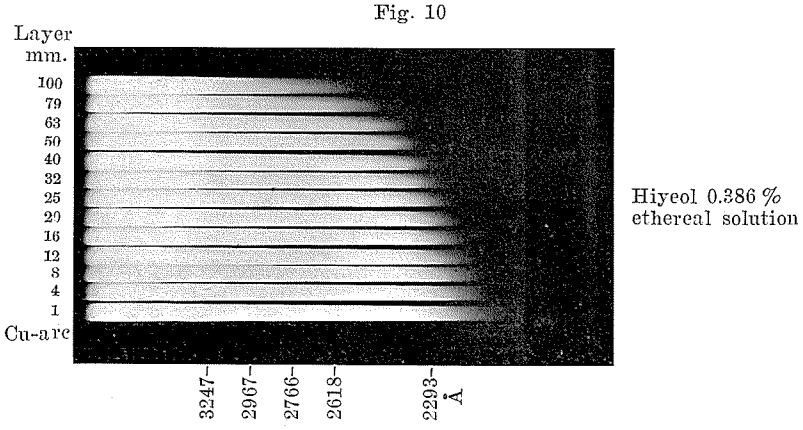
		Molecular weight
$C_{32}H_{54}O$	requires	454
$C_{31}H_{52}O$	„	440
$C_{30}H_{50}O$	„	426
$C_{29}H_{48}O$	„	412
$C_{28}H_{46}O$	„	398

Examination of Absorption Spectra

The absorption spectra of hiyeol in ethereal solution were examined with an ADAM HILGAR E type quartz spectrograph using a hydrogen GEISSLER tube as the source of illumination and the general absorption shown as indicated in the figure.

Judging from the above described results of the analysis and determination of the molecular weight, the differences of numerical value between hiyeol and crusgallin being within the experimental error, these two substances might be isomeride.

Fig. 10



The value of hiyeol, however, was also close to the one of $C_{30}H_{50}O$ or of $C_{29}H_{48}O$.

Separation of Solid and Liquid Fatty Acids

The solid and liquid fatty acids obtained from the oil by saponification were separated by the lead-salt-ether method. The isolation procedure was carried out with the same treatment as detailed in the case of millet (*Panicum miliaceum* L.).

From 2.2 kilograms of the natural oil 1.5 kilograms of liquid fatty acids and 0.16 gram of solid fatty acids were obtained.

Liquid Fatty Acids

To isolate the individual fatty acid from the liquid fatty acids previously obtained, the following experiments were carried out.

The liquid fatty acids were esterified with methyl alcohol, and the methyl esters were then subjected to fractional distillation under diminished pressure. Each fraction was fractionated and thus successive fractional distillations were made five times. The iodine value, refractive index and saponification value of the esters were determined, but satisfactory results could not be obtained. The liquid fatty acids were then distilled under diminished pressure, and passed over almost completely between 186–210°/2 mm. Using this distillate the following bromination and oxidation experiments were made.

a) Bromination:—The bromination procedure of the liquid fatty acids was carried out in the same way as in the case of millet (*Panicum miliaceum* L.).

The bromide thus obtained was treated with petroleum ether to divide it into two portions—soluble and insoluble. The bromides that were insoluble in petroleum ether were then treated with ethyl ether. It was found that all of them were dissolved in it. This furnished evidence that di- and tetra- bromides were present while hexa- or higher bromides were not present in them. The following investigations were made on the bromides which were soluble in petroleum ether and in ethyl ether separately.

(i) The bromides soluble in petroleum ether:—In order to purify these bromides, they were dissolved in a small amount of petroleum ether and placed overnight in the ice-chest. The white crystals thus produced were filtered off. These treatments were repeated several times until no more white crystals were separated out. The yellowish brown bromide in petroleum ether was washed with dilute sodium thiosulphate solution to remove the free bromine. Then to remove the

moisture, it was treated with anhydrous sodium sulphate. The yellowish brown liquid bromide was obtained by removing the petroleum ether, which was analysed with the following results:

Bromine contents (B. C. method),
Subst. 0.4182 g., AgBr 0.3767 g.,
„ 0.4862 „ , „ 0.4413 „ ;

	Neutralisation value	Molecular weight	Bromine contents	
Found	120.1	467.2	38.36,	38.63%
Calc. for $C_{18}H_{34}Br_2O_2$	127.5	440.1	36.15%	

From the above results, the yellowish brown liquid bromide is not considered to be dibromostearic acid, but considering its molecular weight and bromine contents, it seems to be a dibromide contaminated with some compound whose bromine contents are greater than its own.

(ii) The bromide insoluble in petroleum ether but soluble in ether:—The product was recrystallised from ether until it melted constantly at 114–115° and analysed with the following results:

Bromine contents (B. C. method),
Subst. 0.3268 g., AgBr 0.4121 g.
„ 0.3439 „ , „ 0.4299 „ ;

	Neutralisation value	Molecular weight	Bromine contents	
Found	95.9	585.1	53.67,	53.22%
Calc. for tetrabromostearic acid	93.5	599.9	53.28%	

From the above table, white crystalline bromide which is insoluble in petroleum ether but soluble in ether is shown to be tetrabromostearic acid. The liquid fatty acids therefore contain linolic acid.

b) Oxidation:—The oxidation procedure of liquid fatty acids was performed in the same way as in the case of the one of millet according to Hazura's method. The oxidised white mass thus secured was dried in a $CaCl_2$ desiccator under diminished pressure. To remove unoxidised oil, the white dried mass was treated with petroleum ether, using SOXHLET's apparatus. Then the oxidised fatty acids thus obtained were extracted with ether for 6 days to obtain a compound soluble in ether, which was recrystallised from alcohol, melting at 131–132°, and analysed with the following results:

Subst. 0.1018 g., CO_2 0.2548 g., H_2O 0.1016 g.;

	Neutralisation value	Molecular weight	C%	H%
Found	176.7	317.6	68.26	11.17;

Calc. for dihydroxy-stearic acid	177.4	316.3	68.29	11.47.

The compound thus obtained coincides with dihydroxystearic acid although its melting point is a little lower than that given in the literature. This fact gives evidence of the presence of oleic acid. Next, the oxidised substance insoluble in ether was recrystallised from alcohol and two substances obtained one melting at 156–157° and the other at 172–173°.

Considering the above bromination and oxidation experiments, the following conclusions follow.

(1) Linolenic acid or higher fatty acids are not present in the liquid acid.

(2) The liquid fatty acids are composed largely of linolic, isolinolic and oleic acids.

Solid Fatty Acids

To isolate the individual fatty acid from the mixed solid ones, the following procedure were applied. At first a fractional precipitation from their alcohol solution was carried out by adding a small amount of water at a time, but satisfactory results were not given. Fractional precipitation of magnesium salts was next made with unsatisfactory results. Then the solid fatty acids were subjected to fractional distillation under 2 mm. pressure. The neutralisation values of each distillate were measured and those distillates which had nearly equal values were united and fractionated. This successive treatment was carried out six times. Each distillate was recrystallised from 70 per cent. alcohol, when it was separated in a colourless scale like crystals, melting at 62–62.5°. When mixed with an authentic specimen of palmitic acid, KAHLBAUM "zur Analyse", no change in melting point was observed. Its neutralisation value was 218.9 and the results of the analysis were as follows:

Subst. 0.1022 g., CO_2 0.2814 g., H_2O 0.1410

Found	C 75.09;	H 12.61%;
Calc. for $C_{16}H_{32}O_2$	„ 75.00;	„ 12.50 „.

From the above mentioned facts, the solid fatty acids proved to consist chiefly of palmitic acid.

The author desires to express his great indebtedness to Prof. T. TADOKORO in the Hokkaido Imperial University for his encouragement, to Mr. T. KIKUCHI, the chief engineer of the Hōnen Oil Co., Ltd. for extracting the oils, and to the Department of Education for grants

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